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Antifungal and Antibacterial Activities of Diarylamidine Derivatives

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The antifungal and antibacterial properties of a series of 79 diarylamidine derivatives were evaluated. Several of these compounds exhibited considerable antimicrobial potency. A survey of the structure-activity relationship demonstrated that minor structural variations resulted in significant changes of antimicrobial activity. In general, the structural features required for antifungal activity coincided with those required for antibacterial activity. Both the antifungal and the antibacterial properties of the diarylamidines depended on the presence and the positions, of both amidino groups, on the nature of the central bridge connecting the two aryl moieties, and on the nature of these aryl residues (preferably indole). The most active compound was evaluated for its activity against *Candida albicans* infection in mice.

Investigations on therapeutic use of synthetic diarylamidines led to the discovery of the trypanocidal drugs stilbamidine, pentamidine, and Berenil (2, 13, 16). Some early results (9, 11, 14–16) pointed out that such trypanocidal diarylamidines might also be endowed with antibacterial and antifungal activities. It also became evident that the activities against microorganisms may vary upon slight modification of the molecular structure of the diarylamidines, thus making feasible the development of more specific, chemotherapeutically valuable antimicrobial compounds.

We evaluated the antibacterial and antifungal properties of a large series of diarylamidine derivatives and assessed the structural parameters that underlie their growth-inhibitory activity. The most active compound of this series was further examined for its in vivo efficacy against generalized *Candida albicans* infection in mice.

MATERIALS AND METHODS

Species. Antifungal potency was evaluated with clinical isolates of *Candida albicans*, *C. parapsilosis*, *C. guilliermondii*, *Geotrichum candidum*, *Trichosporum cutaneum*, *Scopulariopsis brevicaulis*, *Microsporum vanbreuseghemii*, *Aspergillus fumigatus*, *A. niger*, and *A. flavus*. Antibacterial activities were estimated by using *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* NCTC 8236, *Streptococcus faecalis* ATCC 8043, *Escherichia coli* NCIB 8743, *Pseudomonas aeruginosa* (clinical isolate), and *Proteus mirabilis* (clinical isolate).

Culture conditions. Fungal strains were grown (27°C) on a complex medium (1), and bacteria were cultured (37°C) on tryptic soy agar (Difco Laboratories, Detroit, Mich.). For bioassays all strains were inoculated on petri dishes (9-cm diameter) containing a well-defined medium consisting of a minimal medium (18) supplemented with amino acids and vitamins, including (in milligrams per liter) L-alanine (8.9), L-arginine (6.3), L-asparagine (1.5), L-aspartic acid (1.3), L-cystine (1.2), L-glutamic acid (1.47), L-glutamine (14.6), glycine (0.75), L-histidine (2.1), L-isoleucine (2.6), L-leucine (2.6), L-lysine (3.6), L-methionine (0.75), L-phenylalanine (1.65), L-proline (1.15), L-serine (1.0), L-threonine (2.38), L-tryptophan (0.51), L-tyrosine (1.8), L-valine (1.8), D-calcium pantothenate (0.1), choline chloride (0.1), folic acid (0.1), *meso*-inositol (0.2), nicotinamide (0.1), pyridoxal hydrochloride (0.1), riboflavin (0.01), and thiamine hydrochloride (0.1). The agar contained also the compound to be tested.

Chemicals. The diarylamidine derivatives listed in Tables 1 through 5 were prepared according to methods already described. The compounds (and references describing their syntheses) were: 1–4, 7, 24 (3); 9, 17, 9, 17, 19–21, 62–64, 70, 75, 78 (4); 12–15, 22, 23 (6); 25 (2); 28–32, 46, 56 (5); 36–38, 42, 43, 49, 52–54, 79 (7); 26 (R. Brodersen, H. Loewe, and H. Ott, U.S. patent 2,838,485, June 1958); 27 (G. Newbery and A. P. T. Easson, U.S. patent 2,394,003, February 1946); 33, 34 (Dr. A. Wander A.G., Chem. Abstr. **64**:5102, 1966); 6, 16, 41 (G. Volz, Justus Liebigs Ann. Chem., manuscript in preparation); 8, 18, 47, 57–61, 77 (J. Ruff, Ph.D. thesis, University of Erlangen, Erlangen, Federal Republic of Germany); 10, 11, 44, 45, 48, 66–69, 71, 73, 74 (H. Griesmeier, Ph.D. thesis, University of Erlangen, 1979); 35 (R. Schlee, Ph.D. thesis, University of Erlangen, in preparation); 39, 40, 50, 51, 65, 71, (H. Char, Ph.D. thesis, University of Erlangen, 1978); 55, (H. P. Wolff, Ph.D. thesis, University of Erlangen, 1977); 76 (E. Walkenhorst, Ph.D. thesis, University of Erlangen, 1970).

For comparative purposes, some commercially available antifungal substances, such as nystatin (Labaz A. G., Basel, Switzerland), amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.), pimaricin (Pimafucin; Mycofarm, Delft, The Netherlands),

5-fluorocytosine (Hoffmann-La Roche & Co., A. G., Basel, Switzerland), and 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1*H*-imidazole (miconazole; Janssen Pharmaceutica, Beerse, Belgium), were included in the antifungal assays.

Susceptibility testings. For estimation of minimal inhibitory concentrations, serial dilutions (1:3) of the compounds were prepared in sterile distilled water, and 1-ml amounts of the dilutions were mixed with 9 ml of medium and transferred to petri dishes. The plates were then inoculated with about 10^3 colony-forming units of the fungal or bacterial strain. For the bacteria the results were recorded after an overnight incubation (37°C), and for the fungi readings were made after incubation at 27°C for 3 days.

The minimal inhibitory concentrations were defined as the lowest concentrations of compound inhibiting macroscopically visible growth of fungi or bacteria.

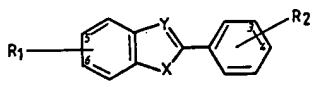
In vivo tests. Four- to six-week-old female NMRI (Naval Medical Research Institute) mice weighing 18 g were used in in vivo tests. They were grouped in 12s and inoculated intravenously with 0.2 ml of a *C. albicans* suspension, containing 7×10^4 CFU, per mouse. The *C. albicans* cells were grown overnight at 37°C, collected, and suspended in saline. Cells were counted after plating and incubation overnight (37°C); meanwhile, cell suspensions intended for injection were kept at 4°C.

Solutions of compound 56, miconazole, and amphotericin B were prepared in 5% (wt/vol) glucose and administered intraperitoneally at 0.2 ml once daily for 5 consecutive days starting on the day of infection. Control mice received 0.2 ml of a 5% (wt/vol) glucose solution. The number of deaths was recorded daily for 30 days.

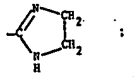
RESULTS

To facilitate the discussion on the structure-activity relationship, the compounds tested were divided into five classes (A, B, C, D, and E) according to the type of the amidino-substituted skeleton. Classes A, C, and D were subdivided according to the type of the heterocyclic ring (indole, benzofuran, benzo[*β*]thiophene, indene, or benzimidazole). Class B compounds contained those compounds where each of the amidino-substituted rings was a benzene. Some miscellaneous compounds were brought together in class E. For each type we considered the influence of heteroatoms in the skeleton, the effect of the positioning of the amidino groups, the role of substituents in the bridges connecting the ring systems, and the role of the nature of the cationic groups.

TABLE 1. *Class A compounds and their minimal inhibitory concentrations (MICs)*



Compound no.	X	Y	R_1	R_2	MIC (μg/ml)									
					a	b	c	d	e	f	g	h	i	j
I. Indoles														
1	NH	CH	Am* (5)	Am (3)	>60	>60	3	>60	3	>60	60	>60	10	-***
2	NH	CH	Am (5)	Am (4)	30	60	3	3	3	10	10	60	3	30
3	NH	CH	Am (6)	Am (3)	>60	60	10	>60	>60	10	>60	>60	10	30
4 (DAPI)	NH	CH	Am (6)	Am (4)	10	3	1	3	1	3	3	>60	3	-
5	NH	CNH_2	Am (6)	Am (4)	>60	>60	30	30	30	>60	>60	>60	>60	>60
6	NH	CH	Im** (6)	Im (4)	>60	3	3	>60	3	10	30	>60	60	60
II. Benzofurans														
7	O	CH	Am (5)	Am (4)	3	30	1	10	3	10	60	>60	>60	>60
8	O	CH	Im (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
9	O	CH	Am (5)	NH_2 (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
10	O	CH	NH-C(=NH)-NH2 (5)	NH-C(=NH)-NH2 (4)	60	60	10	30	10	10	60	>60	60	-
11	O	CH	N=CH-N(CH3)2 (5)	N=CH-N(CH3)2 (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
III. Benzo[b]thiophenes														
12	S	CH	Am (5)	Am (3)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
13	S	CH	Am (5)	Am (4)	60	>60	1	10	3	10	10	>60	>60	>60
14	S	CH	Am (6)	Am (3)	>60	>60	10	>60	>60	>60	>60	>60	>60	>60
15	S	CH	Am (6)	Am (4)	10	>60	10	10	3	10	>60	>60	>60	>60
16	S	CNH_2	Am (5)	Am (4)	30	-	10	10	-	-	30	>60	-	-
17	S	CH	Im (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
18	S	CH	Im (6)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
19	S	CH	NH-C(=NH)-NH2 (5)	NH-C(=NH)-NH2 (4)	10	10	3	10	3	10	10	60	3	30
20	S	CH	NH-C(=NH)-NH2 (6)	NH-C(=NH)-NH2 (4)	10	-	10	30	-	-	10	60	-	-
21	S	CH	CH=N-NH-C(=NH)-NH2 (6)	CH=N-NH-C(=NH)-NH2 (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
22	SO_2	CH	Am (5)	Am (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
23	SO_2	CH_2	Am (5)	Am (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
IV. Indene														
24	CH_2	CH	Am (6)	Am (4)	3	10	1	3	3	1	3	>60	3	10

*Am = amidino = —C(=NH)—NH ; **Im = imidazolino =  ; ***not tested

a : Candida albicans; b : Candida guillermondii; c : Candida parapsilosis ; d : Aspergillus fumigatus; e : Aspergillus niger; f : Aspergillus flavus; g : Trichosporum cutaneum; h : Geotrichum candidum; i : Scopulariopsis brevicaulis; j : Microsporum vanbreuseghemii.

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peritoneally at 0.2 ml once daily for
starting on the day of infection.
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type we considered the influ-
ms in the skeleton, the effect
of the amidino groups, the role
the bridges connecting the ring
ole of the nature of the cationic

centrations (MICs)

(μg/ml)				
f	g	h	i	j
>60	60	>60	10	-***
10	10	60	3	30
10	>60	>60	10	30
3	3	>60	3	-
>60	>60	>60	>60	>60
10	30	>60	60	60
10	60	>60	>60	>60
>60	>60	>60	>60	>60
>60	>60	>60	>60	>60
10	60	>60	60	-
>60	>60	>60	>60	>60
>60	>60	>60	>60	>60
10	10	>60	>60	>60
>60	>60	>60	>60	>60
10	>60	>60	>60	>60
-	30	>60	-	-
>60	>60	>60	>60	>60
>60	>60	>60	>60	>60
10	10	60	3	30
-	10	60	-	-
>60	>60	>60	>60	>60
>60	>60	>60	>60	>60
>60	>60	>60	>60	>60
1	3	>60	3	10

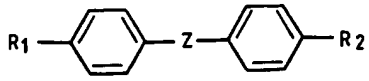
; e : Aspergillus niger; f : Aspergillus
sporum vanbreuseghemii.

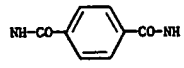
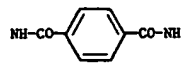
Antifungal activity. The minimal inhibitory concentrations of the compounds, as assessed by plate dilution, are presented in Tables 1 through 5. They correspond to the results obtained for three separate tests. All assays were performed on a synthetic totally defined medium, since antifungal agents may show reduced activity in undefined culture media (10). Results were generally reproducible within a limit of 1 to 3.

Within class A (Table 1) the highest antifungal activity was noted for compound 4 (DAPI) [6-amidino-2-(4-amidinophenyl)indole]. The isosteric replacement of —NH— by —CH_2— (24) did not significantly alter its overall antifungal effect. However, the isosteric replacement of —NH— by —S— (15) or by —O— (2, 7) reduced the activity, and the replacement by —SO_2— (22, 23) even abolished it. The order of diminishing activity depended on the positions of the amidino groups: with —NH—, 6.4′ > 5.4′ > 6.3′ ≈ 5.3′ (4, 2, 3, 1), with —S—, 5.4′ > 6.4′ > 6.3′ ≈ 5.3′ (13, 15, 14, 12). Substitution of an extra amino group in position 3 decreased the activity (compare 5 with 4 and 16 with 13). On substituting imidazolino groups for amidino groups, the antifungal activities of type A compounds generally diminished. This detrimental effect was less pronounced with the more active indoles (6, 4) than with the benzofurans (8, 7) or benzothiophenes (17, 13). Less disadvantageous was the replacement of the amidino groups by guanidino groups, as shown by 10 and 7 and by 19 and 13. The antifungal activity was abolished if one amidino group (7) was replaced by an amino group (9) or if both (7) were replaced by dimethylformamidino (11) or if both (15) were replaced by amidinohydrazone (21).

The bridge connecting the two benzene rings in type B (Table 2) could be linear as well as cyclic. The decreasing order of antifungal activ-

TABLE 2. *Class B compounds and their minimal inhibitory concentrations (MICs)*



Compound no.	Z	R_1	R_2	MIC (μg/ml) a	b	c	d	e	f	g	h	i	j
25 (Stilbamidine isethionate)	CH=CH	Am*	Am	3	1	1	3	3	3	10	>60	10	30
26 (Berenil)	NH-N=H	Am	Am	10	30	3	10	3	30	30	60	60	30
27 (Pentamidine isethionate)	O-$(CH_2)_5$-O	Am	Am	3	3	10	>60	3	>60	1	>60	>60	10
28		Am	Am	10	10	1	1	1	1	1	10	3	3
29		Am	Am	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
30		Am	Am	>60	>60	3	3	3	30	>60	>60	10	-***
31		Am	Am	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
32		Am	Am	60	3	30	3	3	30	>60	>60	>60	>60
33		Am	Am	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
34		Im**	Im	>60	30	60	1	30	>60	>60	>60	>60	>60

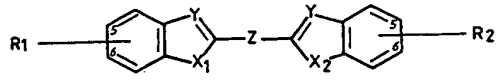
*, **, ***; a to j : see footnotes to Table 1.

ity for the linear bridges, —CH=CH— (25) > —N=N—NH— (26) ≈ —O—$(CH_2)_5$—O— (27), did not reveal any positive influence of heteroatoms, whereas for the five-membered heterocyclic bridges the order pyrrole (28) > furan (30) ≈ thiophene (32) > imidazole (29) ≈ oxdiazole (31) indicated the beneficial influence of the heteroatomic —NH— link in the pyrrole ring. The two representatives (33 and 34) of the Phthalanilides (Dr. A. Wander A. G., Bern, Switzerland) demonstrated the curious, but as yet unexplained, fact that in this series the imidazolino groups generally conferred a much higher biological activity than did the amidino groups.

Type C compounds (Table 3) consisted of two separated heterocyclic ring systems connected by an alkyl chain or another sort of bridge. The order of decreasing antifungal activity was benzofuran (38) ≈ indole (50, 35) > benzothiophene (53) ≈ benzimidazole (54). When the positions of the two amidino groups were altered in one or both benzofuran moieties (or indole moieties), the order of decreasing activity was 5.5′ (38, 50), 5.6′ (39, 51), 6.6′ (40). The addition of a methyl group in position 3 of the benzofuran ring abolished the activity (41, 38). The insertion of a double bond between a benzofuran moiety and an indole moiety was not advantageous (49, 50). Likewise, the antifungal activity decreased after insertion of two or three conjugated double bonds between the heterocyclic ring systems (38, 44, 45) or after introduction of a methyl group in the double bond of the central chain (42, 38). However, some activity was regained upon hydrogenation of the central chain (43, 42). A decrease in activity was noted upon hydrogenation of the double bond of the central chain (37, 38), and all activity was gone if the central chain was shortened to —CH_2— (36). An imidazole ring as bridge (46) abolished the activity, whereas replacement of the amidino groups by imidazolino groups brought about a reduction in activity (47, 38; 48, 44).

Type D compounds (Table 4) consisted of a heterocyclic system linked by a bridge to a benzene ring moiety. Within this class (as with the other classes) the indole appeared to be superior over the benzofuran ring system (55, 62). In benzofurans a bridge composed of two conjugated double bonds (67) was better than one of three (68) or only one (62) double bond. An extra methyl group (64, 62) or hydrogenation of the double bond (63, 62) had a relatively mild

TABLE 3. *Class C compounds and their minimal inhibitory concentrations (MICs)*



Compound no.	X_1	Y	X_2	Z	R_1	R_2	MIC (μg/ml) a	b	c	d	e	f	g	h	i	j
I. Indole/Indole																
35	NH	CH	NH	CH=CH	Im** (6)	Im (6)	>60	10	10	3	3	30	30	>60	30	3
II. Benzofuran/Benzofuran																
36	O	CH	O	CH_2	Am* (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
37	O	CH	O	$(CH_2)_2$	Am (5)	Am (5)	3	3	>60	>60	>60	>60	30	>60	>60	>60
38	O	CH	O	CH=CH	Am (5)	Am (5)	3	1	3	10	1	10	>60	10	30	>60
39	O	CH	O	CH=CH	Am (5)	Am (6)	30	30	30	30	>60	>60	30	>60	>60	>60
40	O	CH	O	CH=CH	Am (6)	Am (6)	60	60	>60	>60	>60	>60	>60	>60	>60	>60
41	O	CCH_3	O	CH=CH	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
42	O	CH	O	$CH=CCH_3$	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
43	O	CH	O	CH_2-$CHCH_3$	Am (5)	Am (5)	3	1	>60	>60	>60	>60	3	>60	>60	>60
44	O	CH	O	$(CH=CH)_2$	Am (5)	Am (5)	10	10	30	10	>60	>60	>60	>60	>60	>60
45	O	CH	O	$(CH=CH)_3$	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
46	O	CH	O		Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
47	O	CH	O	CH=CH	Im (5)	Im (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
48	O	CH	O	$(CH=CH)_2$	Im (5)	Im (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
III. Benzofuran/Indole																
49	O	CH	NH	-	Am (5)	Am (5)	10	10	1	3	1	3	10	>60	3	>60***
50	O	CH	NH	CH=CH	Am (5)	Am (5)	30	3	3	3	60	60	3	>60	>60	>60
51	O	CH	NH	CH=CH	Am (5)	Am (6)	>60	3	>60	10	>60	>60	>60	>60	>60	>60
IV. Benzofuran/Benzo(8)thiophene																
52	O	CH	S	CH=CH	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
V. Benzo(8)thiophene/Benzo(8)thiophene																
53	S	CH	S	CH=CH	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
VI. Benzimidazole/Benzimidazole																
54	NH	N	NH	CH=CH	Am (5)	Am (5)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60

*, **, ***, a to j : see footnotes to Table 1.

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. Likewise, the antifungal activity
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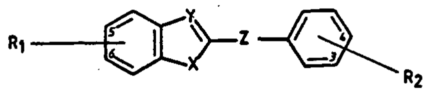
38, 44, 45) or after introduction of
) in the double bond of the central
. However, some activity was re-
ydrogenation of the central chain
rease in activity was noted upon
of the double bond of the central
and all activity was gone if the
was shortened to $-CH_2-$ (36)
ing as bridge (46) abolished the
as replacement of the amidino
lazolino groups brought about a
tivity (47, 38; 48, 44).
pounds (Table 4) consisted of a
stem linked by a bridge to a ben-
ty. Within this class (as with the
he indole appeared to be superior
ofuran ring system (55, 62). In
bridge composed of two conju-
onds (67) was better than one of
only one (62) double bond. An
oup (64, 62) or hydrogenation of
d (63, 62) had a relatively mild

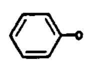
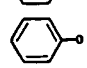
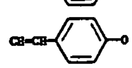
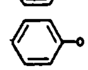
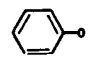
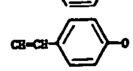
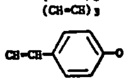
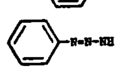
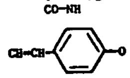
concentrations (MICs)

- R2

MIC (ug/ml)					
e	f	g	h	i	j
3	30	30	>60	30	3
>60	>60	>60	>60	>60	>60
>60	>60	30	>60	>60	>60
1	10	>60	10	30	>60
>60	>60	30	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	3	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
1	3	10	>60	3	-***
60	60	3	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60
>60	>60	>60	>60	>60	>60

TABLE 4. *Class D compounds and their minimal inhibitory concentrations (MICs)*



Compound no.	X	Y	Z	R_1	R_2	MIC (ug/ml) a	b	c	d	e	f	g	h	i	j
I. Indoles															
55	NH	CH	CH=CH	Am* (6)	Am (4)	3	3	3	3	3	3	3	10	3	3
56	NH	CH		Am (6)	Am (4)	3	0.3	0.3	3	1	1	1	3	1	3
57	NH	CH		Am (6)	Am (3)	10	3	3	3	3	10	3	60	10	3
58	NH	CH		Am (6)	Am (4)	10	3	10	>60	>60	>60	30	>60	>60	>60
59	NH	CH		Im** (6)	Im (3)	10	3	10	>60	>60	>60	30	>60	>60	>60
60	NH	CH		Im (6)	Im (4)	3	3	3	10	3	10	3	10	3	10
61	NH	CH		Im (6)	Im (4)	30	3	10	60	>60	>60	60	>60	>60	>60
II. Benzofurans															
62	O	CH	CH=CH	Am (5)	Am (4)	30	30	3	>60	>60	>60	10	>60	>60	>60
63	O	CH	$(CH_2)_2$	Am (5)	Am (4)	30	30	10	>60	>60	>60	3	>60	60	>60
64	O	CH	$CH=CCH_3$	Am (5)	Am (4)	60	60	30	>60	>60	>60	10	>60	>60	>60
65	O	CH	CO-NH	Am (5)	Am (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
66	O	CH	CH=N-NH	Am (5)	Am (4)	30	>60	60	3	10	60	3	>60	-***	-
67	O	CH	$(CH=CH)_2$	Am (5)	Am (4)	1	1	1	10	10	10	10	>60	10	-
68	O	CH	$(CH=CH)_3$	Am (5)	Am (4)	30	3	>60	>60	>60	>60	>60	>60	-	-
69	O	CH		Am (5)	Am (4)	>60	3	>60	>60	>60	>60	10	>60	>60	>60
70	O	CH		Am (5)	Am (4)	>60	30	>60	>60	>60	>60	>60	>60	-	-
71	O	CH	CH=CH	Im (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
72	O	CH	$(CH=CH)_2$	Im (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
73	O	CH	CO-NH	Am (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
74	O	CH		Im (5)	Im (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
III. Benzo(β)thiophene															
75	S	CH	CO-NH	Am (5)	Am (4)	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
IV. Benzimidazole															
76	NH	N	NH	Am (5)	Am (4)	>60	60	10	>60	10	>60	>60	>60	60	30

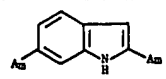
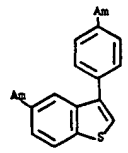
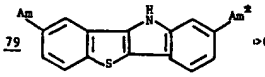
*, **, ***; a to j : see footnotes to Table 1.

effect. The introduction of nitrogen in the bridge did not afford much advantage, as evidenced by the poor activity of compounds 70, 73, and 76. The most beneficial influence was observed by "phenoxy-extension" (56). The optimal position for the cationic group on the benzene ring appeared to be 4′ (56, 57; 60, 59). Addition of a double bond to the phenoxyextension in the bridge decreased the activity (58, 56; 61, 59). Replacement of amidino groups by imidazolino groups diminished the antifungal activity (60, 56; 59, 57; 61, 58; 71, 62; 72, 67), which is in line with the observations made with the type A and type C compounds.

The findings obtained for the miscellaneous compounds (type E) (Table 5) demonstrated that the skeleton for the two cationic groups should not be too short (77) or too rigid (79) or angular (78) (as opposed to linear [13]).

Antibacterial activity. The structure-activity relationship for antibacterial activity of type A compounds (Table 6) roughly paralleled the observations for antifungal activity (Table 1 through 5). The guanidino groups appeared

TABLE 5. *Miscellaneous compounds and their minimal inhibitory concentrations (MICs)*

Compound no.	Structure	MIC (μg/ml) a	b	c	d	e	f	g	h	i	j
77		>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
78		>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
79		>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
Reference compounds											
80	5-fluorocytosine	0.1	0.1	0.1	>60	1	>60	0.1	1	-***	-
81	Nystatin	1	1	1	1	1	1	1	1	-	-
82	Pimafucin	10	10	10	10	10	>60	10	30	-	-
83	Amphotericin B	0.1	0.1	0.1	0.3	0.1	0.3	0.1	0.3	-	-
84	Miconazole	0.03	0.3	0.3	1	0.3	1	0.3	1	<0.01	-

*, ***; a to j : see footnotes to Table 1.

quite favorable for antibacterial activity (20, 15; 19, 17, 13; 10, 8, 7), and whereas isosteric replacement of —S— or —O— by —SO_2— destroyed antifungal activity, some antibacterial activity was maintained (22, 23).

For the type B compounds the same order of antibacterial activity (Table 6) was observed as noted for the antifungal activity (Table 2).

Among type C compounds the antibacterial activity was generally moderate. The parallelism between antibacterial and antifungal activity was evident for the indoles 50, 51, 35 and the benzofurans 38, 39, 47. The unorthodox influence of the methyl group was noticed again (42, 43). Most striking, however, was the fact that compound 49 [5-amidino-2-(5-amidino-benzofuranyl)indole], while prominent as an antifungal agent, did not possess any antibacterial activity. On the other hand, benzimidazole 54—which was not active as an antifungal—exhibited some antibacterial activity.

The parallelism between antifungal activity and antibacterial activity was also observed within the type D compounds, except that the most active antibacterial compound (mainly in respect to *P. mirabilis*) was the imidazolino compound 60, whereas the top antifungal compound was the isosteric amidino compound 56.

In vivo tests. Since compound 56 exhibited the greatest antifungal activity in vitro, it was further evaluated for its efficacy in vivo. Mice were infected intravenously with 7×10^4 colony-forming units of *C. albicans*. This dose caused 100% mortality within 3 weeks, the first dead being observed after 9 days of infection (Fig. 1). For comparative purposes, miconazole and amphothericin B were included at the same concentrations as compound 56, i.e., at 40 and 10 mg/kg for miconazole and at 20 mg/kg for amphotericin B. Compound 56 was tested at 40, 20, and 10 mg/kg.

Preliminary toxicity experiments revealed that when compound 56 was administered at 40 mg/kg, all mice died within 9 days, the mean survival time being 4.33 ± 2.50 days. When compound 56 was injected at 20 mg/kg, 40% of the mice died before day 30, but when injected at 10 mg/kg, compound 56 did not cause casualties. Likewise, no mortality was noted upon intraperitoneal administration of miconazole at 40 mg/kg or amphotericin B at 20 mg/kg.

The protective effect of compound 56, miconazole, and amphotericin B in *Candida*-infected mice is shown in Fig. 1. Amphotericin B was the

hibitory concentrations (MICs)

/ml)				
f	g	h	i	j
·60	>60	>60	>60	>60
60	>60	>60	>60	>60
i0	>60	>60	>60	>60
0	0.1	1	-***	-
1	1	1	-	-
)	10	30	-	-
1	0.1	0.3	-	-
	0.3	1	<0.01	-

e isosteric amidino compound 56.
ıts. Since compound 56 exhibited
ntifungal activity in vitro, it was
ıted for its efficacy in vivo. Mice
intravenously with 7×10^4 colony-
of *C. albicans*. This dose caused
y within 3 weeks, the first dead
l after 9 days of infection (Fig. 1).
ve purposes, miconazole and am-
were included at the same concen-
npound 56, i.e., at 40 and 10 mg/
:ole and at 20 mg/kg for ampho-
pound 56 was tested at 40, 20, and

toxicity experiments revealed
pound 56 was administered at 40
e died within 9 days, the mean
eing 4.33 ± 2.50 days. When com-
njected at 20 mg/kg, 40% of the
e day 30, but when injected at 10
nd 56 did not cause casualties.
ortality was noted upon intraper-
tration of miconazole at 40 mg/
icin B at 20 mg/kg.
'e effect of compound 56, micon-
notericin B in *Candida*-infected
ı Fig. 1. Amphotericin B was the

TABLE 6. *Antibacterial activities of diarylamidine derivatives*

Compound[a]	Minimal inhibitory concentration (μg/ml) for:				
	S. faecalis	*S. aureus*	*E. coli*	*P. mirabilis*	*B. subtilis*
2	30	3	>60	>60	3
3	30	10	>60	>60	30
4	3	3	3	>60	—[b]
5	60	10	60	>60	—
6	60	10	10	>60	—
7	>60	60	>60	>60	30
10	60	30	>60	>60	30
12	>60	30	>60	>60	>60
13	10	30	>60	>60	30
16	>60	10	>60	>60	—
17	60	10	60	>60	60
19	10	10	60	>60	10
20	10	10	10	>60	10
22	30	10	>60	>60	60
23	30	10	60	>60	—
24	10	3	10	>60	—
25	>60	10	>60	>60	—
26	10	10	>60	>60	30
27	10	3	60	>60	30
28	10	10	10	>60	10
30	10	3	>60	>60	30
32	60	30	10	>60	>60
34	60	3	>60	>60	10
35	10	3	30	>60	—
36	60	10	>60	>60	10
37	60	10	>60	>60	10
38	60	10	>60	>60	10
39	60	30	>60	>60	10
40	10	10	>60	>60	10
43	30	10	>60	>60	10
44	>60	30	>60	>60	30
50	60	10	10	>60	>60
51	60	10	60	>60	—
54	60	10	>60	>60	—
55	10	3	10	>60	—
56	1	1	1	>60	10
57	10	3	60	>60	—
58	10	3	10	60	10
59	3	1	3	>60	3
60	1	1	3	3	1
61	3	1	10	>60	1
62	>60	30	>60	>60	30
63	>60	10	>60	>60	—
64	>60	10	>60	>60	30
65	>60	3	>60	>60	3
67	30	3	>60	>60	10
68	>60	10	>60	>60	10
69	>60	10	>60	>60	10

TABLE 6—continued

Compound[a]	Minimal inhibitory concentration (μg/ml) for:				
	S. faecalis	*S. aureus*	*E. coli*	*P. mirabilis*	*B. subtilis*
70	30	10	30	>60	—
74	>60	30	>60	>60	10
75	>60	10	>60	>60	10
76	>60	10	>60	>60	60
80	0.1	0.3	60	60	30
84	>60	<0.3	>60	>60	>60

[a] Compounds not mentioned in the table have minimal inhibitory concentrations of ≥60 μg/ml for all bacterial species tested. *P. aeruginosa* was also included in the antibacterial assay systems, but none of the compounds showed any inhibitory effect on this species at 60 μg/ml (the highest concentration tested).
[b] —, Not tested.

most efficient compound; all mice treated with amphotericin B survived. Miconazole, which was administered below its optimum dose (J. Van Cutsem, personal communication), offered a moderate protection: at 40 mg of miconazole per kg, 35% of the mice survived, and at 20 mg/kg, 25% survived. The protective effect of compound 56 (10 mg/kg) was inferior to those observed with miconazole and amphotericin B. Higher doses of compound 56 could not be used because of its toxic effects. Therefore, one must conclude that compound 56, in spite of its excellent antifungal properties in vitro, does not show much promise as an antifungal agent in vivo.

DISCUSSION

From a series of 79 diarylamidine derivatives divided into five types according to structure, the greatest antifungal activity in vitro was observed with DAPI (4) among type A compounds, stilbamidine isethionate (25) among type B compounds, 1,2-bis-(5-amidino-2-benzofuranyl)ethylene (38) among type C compounds, and 6-amidino-2-[4-(4′-amidinophenoxy)phenyl]indole (56) among type D compounds. Compound 56 was even more active against *Candida* than were nystatin or pimaricin and almost as effective as amphotericin B, 5-fluorocytosine, or miconazole. It showed also good activity against *G. candidum*, *S. brevicaulis* and *M. vanbreuseghemii*. Its imidazolino derivative 6-imidino-2-[4-(4′-imidinophenoxy)-phenyl]indole (60) was most effective against bacteria (especially *P. mirabilis*).

The in vivo tests in mice indicated that although compound 56 showed some protective effect against *Candida*, this compound may be too toxic for systemic use.

A survey of the structure-activity relationship demonstrated that minor structural variations resulted in significant changes of both antifungal and antibacterial activities. These activities depended on the planarity of the molecule, the presence of amidino groups, the nature of the bridge connecting the two aryl moieties (preferably a phenoxy extension) and the nature of the aryl rings (preferably indole).

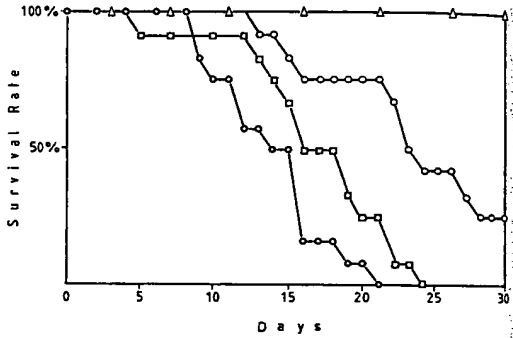


FIG. 1. *Comparison of the protective effects of the heterocyclic diarylamidine compound 56 (□) at 10 mg/kg, amphotericin B (△) at 20 mg/kg, and miconazole (○) at 20 mg/kg in mice infected with 7 × 10⁴ colony-forming units of C. albicans. Infected control mice (○) were treated intraperitoneally with 0.2 ml of a 5% (wt/vol) glucose solution.*

The mechanism by which diarylamidine derivatives exert their antifungal and antibacterial activities may be related to a direct binding of the compounds to nucleic acids, as has been documented in previous studies (12). Since amidino- or guanidino-substituted diaryl derivatives are in general superior in activity to the imidazolino-substituted diaryl derivatives, one may also envisage a direct interaction of the diarylamidines with some proteins (akin to arginine-specific esteroproteases) (17).

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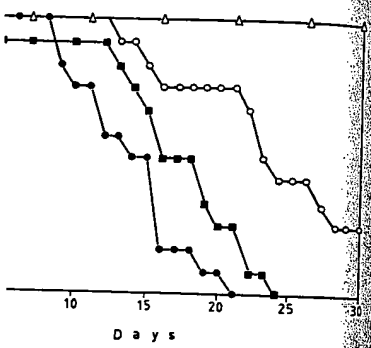
ion (μg/ml) for:

P. mirabilis	*B. subtilis*
>60	—
>60	10
>60	10
>60	60
60	30
>60	>60

entrations of ≥60 μg/ml for all bacterial
y systems, but none of the compounds
ncentration tested).



parison of the protective effects of the urylamidine compound 56 (■) at 10 ericin B (△) at 20 mg/kg, and micon- mg/kg in mice infected with 7 × 10⁴ units of C. albicans. Infected control eated intraperitoneally with 0.2 ml of ucose solution.

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midino groups, the nature of the
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extension) and the nature of the
erably indole).
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be related to a direct binding of
s to nucleic acids, as has been
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ed diaryl derivatives, one may
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ith some proteins (akin to argi-
eroproteases) (17).

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